

DATA EVALUATION RECORD
FORAGING ACTIVITY AND MORTALITY- HONEY BEES AND BUMBLEBEES
Apis mellifera and Bombus terrestris
(NON-GUIDELINE STUDY)

1. **CHEMICAL**: Imidacloprid PC Code No.: 129099

2. **TEST MATERIAL**: Imidacloprid® WG 70 Purity: 70%

3. **CITATION**

Authors: Maus, Ch., R. Schoening and J. Doering

Title: Assessment of effects of a drench application of Imidacloprid® WG 70 to shrubs of *Rhododendron* sp. and to *Hibiscus syriacus* on foraging activity and mortality of honeybees and bumblebees under field conditions

Study Completion Date: January 29, 2007

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Sponsor: Bayer CropScience AG, Environmental Science, Lyon, France

Laboratory Report ID: G201809

MRID No.: 473034-06

DP Barcode: D348269

4. **REVIEWED BY**: John Marton, Staff Scientist, Cambridge Environmental Inc.

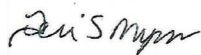
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Date: 05/28/08

5. **APPROVED BY**: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

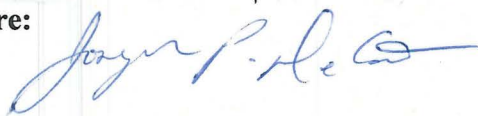
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Date: 06/04/08

6. **APPROVED BY**: Joseph DeCant, Ecologist, EPA/OPP/ERB V

Signature:



Date: 07/01/14

7. STUDY PARAMETERS

Test Species: *Apis mellifera* and *Bombus terrestris*

Age of Test Organisms at Test Initiation: Not specified for bees. *Rhododendron* shrubs were approximately 7 years old; *Hibiscus syriacus* shrubs were approximately 5 years old; the age of the surrounding ornamental plants was not specified

Test Duration: Bees were observed on 10 different days, blossom samples were collected from *Rhododendron* shrubs 35 days after treatment and from *Hibiscus syriacus* shrubs 106-117 days after treatment.

8. CONCLUSIONS:

Honey bees were found foraging more on the ornamental plants than on either the *Rhododendron* or *Hibiscus* plants. More bumblebees were found on the *Rhododendron* than on the ornamentals, but during the *Hibiscus* phase, ornamentals were favored. Honey bees and bumblebee typically preferred the control plants over the treated plants. Residues of imidacloprid and its hydroxy- and olefin-degradates were found in all treated blossoms and in all dead bees including bees from control areas. These data suggest the possibility that the substantial difference in foraging on control plants relative to treated plants for both honeybees and bumblebees also suggests that the residue levels exhibited in this study may deter foraging on the treated plants.

The highest mortality (27 honey bees) was associated with treated rhododendrons where imidacloprid residues in blossoms were as high as 0.79 mg ai/kg (35 days after treatment; DAT) and imidacloprid residues detected in dead bees were as high as 0.091 mg ai/kg (39 – 49 DAT). The highest mortality among bumble bees (14 dead) was associated with treated hibiscus where imidacloprid residues in blossoms were as high as 5.01 mg ai/kg (106 – 117 DAT) and residues in dead bees were as high as 1.663 mg ai/kg (104 – 118 DAT). Average residues in hibiscus blossoms (2.98 mg/kg) 106 – 177 DAT were roughly 10 times those in rhododendron blossoms (0.267 mg/kg) 35 DAT. Residues of the hydroxy degradate were consistently an order of magnitude less than the parent. These data suggest that time is an important parameter when measuring residue levels in blossoms. The residue levels were not measured in *Rhododendron* and *Hibiscus* blossoms at the same time points, so it is impossible to adequately compare levels between the two shrubs. Nonetheless, with equivalent application rates and the substantial difference between residue levels between the two shrubs, it appears that translocation from the soil to the plant requires sufficient time to build up residues in the plants. Furthermore, the longer the time, the greater the residue levels in blossoms where 106 – 117 DAT results in potentially higher residue levels compared to 35 DAT.

Bumble bee colonies (control and treated) used in the rhododendron studies were discovered to have completely died 8 weeks after the study. While the author attributes this to intrinsic factors

related to life span of bumble bees it is unclear whether the bumble bee health was compromised and diminished the capacity of the study to detect potential treatment effects.

9. ADEQUACY OF THE STUDY

A. Classification: Supplemental

B. Rationale: This is a non-guideline study. Dead bees from control plots contained imidacloprid residues indicating that they were foraging in treated plots. The inability of the study to confine bees to treated and control plots limits the utility of the study for documenting potential effects on bees. Additionally, the ability of the study to document actual bee mortality is likely limited.

C. Repairability: N/A

10. **GUIDELINE DEVIATIONS:** This is a non-guideline test.

11. **SUBMISSION PURPOSE:** This study was conducted to determine the effects on foraging activity and mortality of honeybees (*Apis mellifera*) and bumblebees (*Bombus terrestris*) under field conditions after a drenching application with Imidacloprid® WG 70 (70% active ingredient; ai) on shrubs of a hybrid *Rhododendron* species (variety “Roseum Elegans”) and on *Hibiscus syriacus* (variety “Hamabo”) surrounded by an ornamental species composition typically found in suburban gardens in North America. Additionally, the residue levels of imidacloprid and its hydroxy- and olefin-degradates in blossom samples of the rhododendron and hibiscus shrubs were determined in order to demonstrate exposure of the test organisms to imidacloprid and its degradates.

12. MATERIALS AND METHODS

A. Test Organisms

This study was conducted in two different phases. The first consisted of exposing honey bees and bumblebees to treated and untreated *Rhododendron* hybrid species shrubs (variety “Roseum Elegans”, approx. 7 years old). Application rates were 0 (untreated control plants) and 4.3 g ai/m of average plant width. The second phase consisted of exposing honey bees and bumblebees to treated and untreated *Hibiscus syriacus* shrubs (variety “Hamabo”, approx. 5 years old). Application rates were 0 (untreated control) and 4.3 g ai/m plant height. In both phases, ornamental bee-attractive flowering species were placed around and in between the rows of treated

and untreated plants.

One honey bee hive containing 11 combs, approximately 10,000 honey bees and a queen were placed at a distance of 20-25 m from each plot at the beginning of each phase. Two bumblebee colonies containing approximately 100-150 bumblebees each were placed at a distance of approximately 20 m from each plot at the beginning of each phase.

B. Test Design

For the *Rhododendron* and *Hibiscus* phases, each treatment plot consisted of three parallel rows, with each row containing 6 shrubs. *Rhododendron* shrubs were 1.5 m from each other within rows, with 2 m separating the rows. The average shrub height was 1 m and the average shrub width was 1.2 m. *Hibiscus* shrubs were 1 m from each other within rows with 2.5 m separating the rows. The average shrub height was 1m and the width was 0.4 to 0.5 m.

During the *Rhododendron* phase of the study, rows were surrounded by the following ornamentals: *Fragaria* sp., *Pulmonaria officinalis*, *Fuchsia* sp. hybrids, *Centaurea montana*, *Lobelia erinus* and *Lupinus* sp. *Pelagornium* sp. and *Surfinia* sp. were setup between the three rows of *Rhododendron*.

During the *Hibiscus* phase of the study, rows were surrounded by the following ornamentals: *Lavendula angustifolia*, *Calluna vulgaris*, *Centaurea montana*, *Phacelia tanacetifolia*, *Lobelia erinus*, *Helianthus* sp. and *Fragaria* sp. *Pelagornium* sp. and *Surfinia* sp. were setup between the three rows of *Hibiscus*.

For the *rhododendron* phase, no other flowering plants (other than the ornamentals) were reported in the vicinity of the study area. For the *hibiscus* phase though, Mayweed, flowering gladolus, snapdragons and larkspur (20% open blossoms) were in the vicinity of the study area.

Both treated shrub species received 2 liters of test solution by applying the total volume near the stems of the plants without runoff of the water. Application of the test substance to both shrub species was on April 12, 2006.

Foraging activity was assessed for a total of 10 days in the morning and afternoon. During the *Rhododendron* phase, assessments were made from May 21-24, 2006 and from May 28 to June 1, 2006. During the *Hibiscus* phase, assessments were made from July 25 to July 27, 2006, from July 31 to August 4, 2006 and from August 8 to August 9, 2006. Foraging was determined by counting the number of honey bees and bumble bees on the *Rhododendron* or *Hibiscus* plants and in the ornamental rows. Mortality was determined daily by counting the number of dead bees found on linen sheets placed along the ground in between plants and at the entrance to hives/colonies. *Rhododendron* blossom samples were collected on May 17, 2006 (35 DAT) and *Hibiscus* blossom samples were collected on July 27 (Day 106) for controls and July 27 to August

7, 2006, (106 – 117 DAT) for treated. Dead bees and blossom samples were stored at -18°C and stored for residue analysis

Samples of the *Rhododendron* and *Hibiscus* blossoms and the dead bees were analyzed for residues of imidacloprid and its olefin- and hydroxy-degradates using HPLC-MS/MS. The LOQ for imidacloprid and both degradates was 0.010 mg ai/kg for the blossom samples and 0.001 mg ai/kg for the honey bee and bumblebees.

13. REPORTED RESULTS

During the *Hibiscus* phase, honey bee and bumblebee foraging activity was greater on the ornamentals (**Table 1**). During the *Rhododendron* phase, bumblebees preferred the *Rhododendron* shrubs over the ornamentals, while honey bees favored the ornamentals. Honey bees favored the treated ornamentals over the control ornamentals during the *Rhododendron* phase; in all other instances, control plants were favored over the treated ones.

Table 1. Total number of honeybees and bumblebees observed foraging in control and treated plots of rhododendron, hibiscus.

Total Number of Bees Foraging per Plot				
<i>Rhododendron</i> Phase				
Group	Honey bees		Bumblebees	
	Rhododendron	Ornamentals	Rhododendron	Ornamentals
Control	23	64	608	238
Treatment	10	104	107	87
<i>Hibiscus</i> Phase				
Group	Honey bees		Bumblebees	
	Hibiscus	Ornamentals	Hibiscus	Ornamentals
Control	10	192	233	837
Treatment	5	108	9	623

During the *Rhododendron* phase, only two honey bees died in the control group (both died in front of the hive) and no dead bumblebees were found in the control plot (**Table 2**). In the treatment plot, 27 honey bees died, with 25 being found in front of the hive and 2 found within the treated plots. Two bumblebees were found dead (one on the plot and one in front of the hive).

During the *Hibiscus* phase, no dead honeybees were found (**Table 2**). No bumblebees were found dead in the control plot and 14 dead bumblebees were found in the treatment plot, with 12 being found on the plot and 2 being found in front of the hive.

Honey bee hives and bumblebee colonies appeared to be in similar condition by the end of the

exposure period.

Table 2. Total honeybee and bumblebee mortality in plot and at hive for control and treated rhododendron and hibiscus areas.

Total Number of Dead Bees per Plot				
<i>Rhododendron Phase</i>				
<u>Group</u>	Honey bees		Bumblebees	
	On the Plot	By Hive	On the Plot	By Hive
Control	0	2	0	0
Treatment	2	25	1	1
<i>Hibiscus Phase</i>				
<u>Group</u>	Honey bees		Bumblebees	
	On the Plot	By Hive	On the Plot	By Hive
Control	0	0	0	0
Treatment	0	0	12	2

No residues were detected in *Rhododendron* or *Hibiscus* blossoms collected from the control plots (**Table 3**). Residues of imidacloprid and both metabolites were detected in treated blossoms as well as in honey bees and bumblebees found dead in the treated plots during both phases of the study.

No residues were detected in *Rhododendron* or *Hibiscus* blossoms collected from the control plots (**Table 3**). Residues of imidacloprid and both degradates were detected in treated blossoms as well as in honey bees and bumblebees found dead in the treated plots during both phases of the study. Maximum residues of imidacloprid in rhododendron (35 DAT) and hibiscus blossoms (106 – 117 DAT) were 0.79 mg/kg and 5.01 mg/kg, respectively. Maximum imidicloprid residues measured in dead honey bees and bumble bees were 0.022 mg/kg and 0.091 mg/kg, respectively, for rhododendrons; the maximum imidacloprid residue found in dead bumblebees collected around treated hibiscus was 1.663 mg/kg (**Table 3**).

Table 3. Residues of imidacloprid and its hydroxy and olefin-degradates in blossoms and bee samples collected from rhododendrons (Rh) and Hibiscus (H) at specified days after treatment (DAT) with soil drench of 4.3 g ai/m shrub width.

Treatment Group	Sample	Study Part	DAT	Imidacloprid (mg ai/kg)	Hydroxy-degradate (mg ai/kg)	Olefin-degradate (mg ai/kg)
Control	Blossoms	Rh	35	<LOQ	<LOQ	<LOQ
Treatment	Blossoms	Rh	35	0.09-0.79	0.01-0.04	<LOQ-0.01
Control	Blossoms	H	106	<LOQ	<LOQ	<LOQ
Treatment	Blossoms	H	106-117	0.76-5.01	<LOQ-0.45	<LOQ-0.33
Control	2 Honey Bees (colony)	Rh	47	0.005-0.022	<LOQ-0.008	0.001-0.019
Treatment	25 Honey Bees (colony)	Rh	39-49	<LOQ-0.016	<LOQ-0.001	<LOQ-0.001
	2 Honey Bees (plot)	Rh	39-49	0.002-0.091	<LOQ-0.018	<LOQ-0.001
	1 Bumble Bee (colony)	Rh	47	0.001	0.001	0.005
	1 Bumblebee (plot)	Rh	49	0.005	0.003	0.003
Treatment	2 Bumblebees (colony)	H	105	0.003-0.004	0.001-0.003	0.004-0.009
	12 Bumblebees (plot)	H	104-118	0.077-1.663	0.019-0.196	0.031-0.405

Rh- Rhododendron

H- Hibiscus

LOQ- 0.010 mg ai/kg (blossom samples)/ 0.001 mg ai/kg (bee samples)

DAT- Days After Treatment

14. REVIEWER COMMENTS:

The study only describes alternate food pollen/nectar sources available to the bees in close proximity to the study area. Foraging data indicate that honeybees did not appear to forage extensively on either the rhododendrons or hibiscus and only made limited use of the other ornamental plants provided relative to bumblebees. The data indicate that control honey bees contained imidacloprid residues as high as 0.022 mg/kg which is 22 times the LOQ. These data indicate that the honey bees must have foraged in treated areas. Based on the map provided in the report, the control plots were 145 m from imidacloprid treated sites and were well within the distance that bees will typically forage. It is reasonable to believe that bees in treated areas made use of untreated control plants and likely foraged outside of the treatment area as well. The inability of the study to confine bees to treatment and control areas and limit the extent to which the bees fed outside the entire study area limits the utility of the study for detecting potential treatment effects.

The study did not determine the extent to which dead bees could be identified on the linen sheets used to collect this information. Typically, studies examining bee mortality rely on drop zone dead bee traps that have screening to reduce dead bee removal by scavengers. The limited observed bee mortality in this study therefore cannot be construed as actual low mortality. It is apparent though that the majority of dead honey bees were observed near the entrance to the hive; however, for bumble bees the majority of the mortality was observed on the linen sheets between plant rows and not near the entrance to the colony.

Storage stability tests were not reported so it is uncertain whether any effort was made to document the stability of the imidacloprid residues under the study conditions.

Although inspections of the bumble bee colonies 4 weeks after the end of the rhododendron part of the study showed normal foraging and flight behavior in the controls and treated areas, both colonies were dead by 8 weeks. The researchers attributed this loss to “intrinsic factors” (limited normal life span of several weeks to a few months). Bumble bees colonies used in the hibiscus studies were intentionally sacrificed one week after the study and thus it could not be verified whether the loss of colonies in the rhododendron study was due to “intrinsic factors”. The study authors noted that the condition of the colonies after the Rhododendron and Hibiscus studies were similar, and no differences could be found. However, the study is also not able to differentiate between the two treatment groups relative to colony condition due to the close proximity of the treated and control plot and the consequent overlap in foraging areas.

Average residues of imidacloprid and its hydroxy and olefin degradates in rhododendron blossoms (35 DAT) and hibiscus blossoms (106 – 117 DAT) were calculated using the Proc Means procedure of the Statistical Analysis System (SAS Institute, Cary, NC; Release 8.1) and are presented in **Table 4**. Average residues in hibiscus blossoms (2.98 mg/kg) 106 – 177 DAT were

roughly 10 times those in rhododendron blossoms (0.267 mg/kg) 35 DAT. Residues of the hydroxy degradate were consistently an order of magnitude less than the parent.

Table 4. Average (mean \pm standard error) residues of imidacloprid and its hydroxy and olefin degradates in rhododendron (35 days after treatment; DAT) and in hibiscus blossoms (106 to 117 DAT).

Test	Imidacloprid (mg ai/kg)	Hydroxy-degradate (mg ai/kg)	Olefin-degradate (mg ai/kg)
Rhododendron 35 DAT	0.267 \pm 0.043	0.019 \pm 0.002	<LOQ
Hibiscus 106 – 117 DAT	2.98 \pm 0.275	0.292 \pm 0.023	0.214 \pm 0.017

These data suggest that time is an important parameter when measuring residue levels in blossoms. The residue levels were not measured in *Rhododendron* and *Hibiscus* blossoms at the same time points, so it is impossible to adequately compare levels between the two shrubs. Nonetheless, with equivalent application rates and the substantial difference between residue levels between the two shrubs, it appears that translocation from the soil to the plant requires sufficient time to build up residues in the plants. Furthermore, the longer the time, the greater the residue levels in blossoms where 106 – 117 DAT results in potentially higher residue levels compared to 35 DAT.

In spite of the study's limitations the data indicate that in most cases both honey bees and bumble bees preferred to forage on untreated control plants; honey bees foraging on ornamentals adjacent to treated rhododendrons was a notable exception where roughly twice as many bees were found on the ornamentals in treated areas. Except for two honey bees that were found dead near the entrance to control hives associated with rhododendrons, no other mortality was observed for either honeybee or bumble bee controls. The highest mortality (27 honey bees) was associated with treated rhododendrons where imidacloprid residues in blossoms were as high as 0.79 mg ai/kg (35 DAT) and imidacloprid residues detected in dead bees were as high as 0.091 mg ai/kg (39 – 49 DAT).

Overall, the foraging activity of both bumblebees and honeybees on treated shrubs was relatively low. Part of the reason may be due to weather. In the case of honeybees in the *Rhododendron* part of the study, the weather rained on several days, with nearly 100% cloudy conditions on almost all of the days. In this weather, honey bee foraging would likely decline whereas bumblebees are more tolerant of lower temperatures and adverse weather conditions. The weather appeared to be more favorable to foraging in the *Hibiscus* experiment though. And yet foraging by honeybees on both control and treated shrubs was relatively low compared to foraging on the ornamental potted plants in this second phase of the experiment. These data suggest that the ornamental potted plants surrounding the two shrub species played an important role for an alternative source of forage.

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The highest mortality among bumble bees (14 dead) was associated with treated hibiscus where imidacloprid residues in blossoms were as high as 5.01 mg ai/kg (106 – 117 DAT) and residues in dead bees were as high as 1.663 mg ai/kg (104 – 118 DAT).

15. REFERENCES:

No references were provided.

16. STATISTICAL ANALYSIS OF RESIDUE DATA

AVERAGE IMIDACLOPRID, HYDROXY (HYDR) AND OLEFIN (OLEF) RESIDUES IN BLOSSOMS OF 45
RHODODENDRUM (R) AND HIBISCUS (H) 09:40 Sunday, July 13, 2008

Obs	PLANT	_TYPE_	_FREQ_	IMID	HYDR	OLEF	I_SE	H_SE	O_SE
1	H	0	18	2.98222	0.28176	0.21412	0.27465	0.022626	0.017301
2	R	0	18	0.26667	0.01889	.	0.04347	0.002122	.